Published in final edited form as:

Curr Opin Hematol. 2012 July ; 19(4): 273–279. doi:10.1097/MOH.0b013e328353c6bf.

Shp2 function in hematopoietic stem cell biology and leukemogenesis

Sarah C. Nabinger[b] and **Rebecca J. Chan**[a,b]

[a]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana, USA

[b]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana, USA

Abstract

Purpose of review—The protein tyrosine phosphatase Shp2 is encoded by *PTPN11* and positively regulates physiologic hematopoiesis. Mutations of *PTPN11* cause the congenital disorder Noonan syndrome and pathologically promote human leukemias. Given the high frequency of *PTPN11* mutations in human disease, several animal models have been generated to investigate Shp2 in hematopoietic stem cell (HSC) function and leukemic transformation.

Recent findings—Two independent animal models bearing knockout of Shp2 in hematopoietic tissues clearly demonstrate the necessity of Shp2 in HSC repopulating capacity. Reduced HSC quiescence and increased apoptosis accounts for diminished HSC function in the absence of Shp2. The germline mutation Shp2D61G enhances HSC activity and induces myeloproliferative disease (MPD) *in vivo* by HSC transformation. The somatic mutation Shp2D61Y produces MPD *in vivo* but fails to induce acute leukemia, whereas somatic Shp2E76K produces MPD *in vivo* that transforms into full-blown leukemia. HSCs expressing Shp2D61Y do not generate MPD in recipient animals upon transplantation, whereas Shp2E76K-expressing HSCs yield MPD as well as acute leukemia in recipient animals. The mechanisms underlying the unique functions of Shp2D61Y and Shp2E76K in HSC transformation and leukemogenesis continue to be under investigation.

Summary—Further understanding of the physiologic and pathologic role of Shp2 in hematopoiesis and leukemogenesis, respectively, will yield information needed to develop therapeutic strategies targeted to Shp2 in human disease.

Keywords

hematopoietic stem cell; leukemogenesis; *PTPN11*; Shp2

Correspondence to Rebecca J. Chan, MD, PhD, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 W, Walnut Street, R4-W170, Indianapolis, IN 46202, USA. Tel: +1 317 278 2807; fax: +1 317 274 8679; rchan@iupui.edu.

Conflicts of interest

There are no conflicts of interest.

PROTEIN TYROSINE PHOSPHATASE, Shp2

The protein tyrosine phosphatase Shp2 was originally cloned 20 years ago [1,2], and has been extensively studied by multiple investigators at the biochemical, physiological, and pathological levels [3,4,5]. Shp2 was immediately identified as an intriguing signaling molecule due to its two Src homology 2 domains at its aminoterminal end. Shp2 also contains a phosphatase domain, a proline-rich motif, and carboxyl-terminal phosphotyrosyl residues that also contribute to its signaling capacities [4]. Shp2 participates in myriad signaling cascades including the Ras-mitogen-activated protein kinase, the JAK–STAT, and phosphoinositol 3-kinase–AKT pathways, and positively contributes to multiple cellular functions including proliferation, differentiation, cell cycle maintenance, and migration [3,4,6,7]. Although a protein tyrosine phosphatase, which is traditionally thought to inactivate kinases and to serve as a negative regulator of cell function, Shp2 has been shown to promote cell growth and function by both upregulating positive signaling pathways [4,7] and by downregulating negative signaling pathways [6]. Shp2 function depends on its phosphatase as well as it adapter function. Although the physiologic substrate of Shp2 remains uncertain, it is clear that its phosphatase function is necessary to support its full role in response to multiple growth factors and cytokines. On the other hand, loss of phosphatase activity does not void the positive signaling function of Shp2, which has been demonstrated most dramatically in individuals with the congenital disorder LEOPARD syndrome (multiple lentigenes, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth, sensorineural deafness), who bear *PTPN11* mutants lacking phosphatase function [8,9], but are phenotypically similar to individuals with Noonan syndrome, who bear *PTPN11* mutants with elevated phosphatase function [10]. The present review highlights the several animal models used to delineate the functional role of wild-type Shp2 as well as Shp2 gain-of-function mutants in hematopoiesis, hematopoietic stem cell (HSC) biology, myeloproliferative disorder, and acute leukemia.

Shp2 IN NORMAL HEMATOPOIESIS AND HEMATOPOIETIC STEM CELL FUNCTION

Although Shp2 is ubiquitously expressed, its role in hematopoiesis became clear early on and has been studied in greater depth and detail than in any other tissue. Initial studies using murine embryonic stem cells bearing an exon 3 deletion from *PTPN11* resulting in a truncated protein lacking amino acids 46–110 demonstrated impaired embryonic stem cell-derived erythroid and myeloid progenitor development and reduced contribution to hematopoietic tissues in chimeric mice [11,12]. Further studies demonstrated that Shp2 is necessary in the initial steps of embryonic stem cell differentiation [13] and that HSCs from Shp2 heterozygous animals have reduced repopulating units and reduced self-renewal compared with wild-type animals [14]. Collectively these data suggest that Shp2 is important in normal hematopoiesis by promoting differentiation from very early stages of embryonic stem cell development and by supporting HSC function and self-renewal.

Given that animals bearing a germline knockout of Shp2 are embryonic lethal [15], two independent animal models bearing a conditional targeted *PTPN11* allele have been

developed in the labs of Dr Benjamin Neel [16] and Dr Gen-Sheng Feng [17] and crossed with Mx1-Cre-positive transgenic animals, in order to knockout Shp2 expression in hematopoietic tissues upon treatment with polyI : polyC. Both animal models lacking Shp2 expression demonstrated cytopenia in peripheral blood and bone marrow [18■■,19■■]. Upon phenotypic analysis of various hematopoietic cell lineages, absolute numbers of Lin-negative Sca1-positive Kit-positive (LSK) cells, common lymphoid progenitors, common myeloid progenitors (CMP), granulocyte– monocyte progenitors (GMPs), and megakaryocyte–erythroid progenitors (MEPs) were all markedly reduced in both polyI : polyC-treated animal models (Fig. 1a).

To examine HSC function in the absence of Shp2, Zhu *et al*. [19■■] transplanted bone marrow cells from polyI : polyC-treated $Shp2^{flox/flox}$:Mx1-Cre-negative or $Shp2^{flox/flox}$: Mx1-Cre-positive animals into lethally irradiated recipients with wild-type competitor bone marrow cells at a ratio of 1:1 and 4:1. Shp2-deficient HSCs failed to reconstitute lethally irradiated recipients, and Shp2 null cells gave rise to few, if any, blood cells in the 1: 1 ratio. Consistent with the findings using the Shp2 heterozygous mouse model described above [14], these data suggest that Shp2 is important for HSC function *in vivo*. As bone marrow cells from polyI : polyC-treated $Shp2^{flox/flox}$:Mx1-Cre-positive donor mice failed to reconstitute lethally irradiated wildtype recipients, experiments were performed to test the importance of Shp2 on HSC maintenance and expansion following engraftment by treating recipient animals with polyI : polyC after transplantation [19■■]. Bone marrow cells from untreated $Shp2^{flox/flox}$: Mx1-Cre-negative or $Shp2^{flox/flox}$: Mx1-Cre-positive mice were first transplanted into lethally irradiated wild-type recipient animals followed by treatment with polyI : polyC 4 weeks posttransplant. Animals transplanted with $Shp2^{flox/flox}$: Mx1-Cre-negative cells demonstrated similar levels of chimerism regardless of whether the donor mice were treated with polyI : polyC prior to transplantation or whether the recipient mice were treated with polyI : polyC after transplantation. In contrast, whereas recipient animals transplanted with $Shp2^{flox/flox}$:Mx1-Cre-positive cells from poly-I:polyC-treated donors demonstrated very low levels of hematopoietic reconstitution derived from the $Shp2^{flox/flox}$: Mx1-Cre-positive cells, recipient animals transplanted with $Shp2^{flox/flox}$: Mx1-Cre-positive cells and treated with polyI : polyC posttransplantation demonstrated significantly higher levels of $Shp2^{flox/flox}$: Mx1-Cre-positive cell-derived hematopoiesis, although not as high as that achieved with $Shp2^{flox/flox}$: Mx1-Cre-negative cells [19■■]. Chan *et al*. [18■■] performed similar studies by transplanting bone marrow cells from Mx1-Cre-positive; *PTPN11*$^{flox/flox}$, Mx1-Cre-positive; *PTPN11*$^{flox/+}$, and Mx1-Cre-positive; *PTPN11*$^{+/+}$ animals into lethally irradiated recipients and treating recipients with polyI : polyC 5 weeks posttransplantation. Although they observed a reduction of donor chimerism from cells bearing a homozygous deletion of Shp2 similar to that of Zhu *et al*. [19■■], they also found that transplantation of cells bearing a heterozygous deletion of Shp2 (Mx1-Cre-positive; *PTPN11*$^{flox/+}$) yielded chimerism intermediate between the wild-type and the homozygous cells, indicating that Shp2 function in HSCs acts in a gene dosage-dependent manner [18■■] (Fig. 1a).

The finding that transplantation of Shp2 homozygous cells from both untreated animal models initially generated chimerism levels similar to that of transplanted wild-type (i.e.,

Mx1-Cre-negative) cells, with a precipitous drop in chimerism only after polyI : polyC treatment, suggested that HSC homing and initial engraftment depends on Shp2. In order to examine the effects of loss of Shp2 on homing, Zhu *et al*. [19■■] transplanted CFDA-SE-labeled Shp2-deleted or control Lin-negative bone marrow cells into lethally irradiated wild-type mice, and recipients were sacrificed after 16h to quantify CFDA-SE levels in the bone marrow. Animals transplanted with Shp2-deficient cells had significantly reduced CFDA-SE-stained cells compared with animals transplanted with control cells, indicating the need of Shp2 for normal HSC homing. To define a cellular mechanism that may underlie reduced homing and engraftment of Shp2-deficient cells, phenotypically defined HSCs were evaluated for cell cycle status. Studies from both the Neel laboratory [18■■] and the Feng laboratory [19■■] demonstrated that Shp2-deficient cells are less quiescent with reduced cells in G0/G1 stage of the cell cycle (Fig. 1a). Similar results were found in phenotypically defined Shp2 heterozygous HSCs [14]. Taken together, these data support the importance of Shp2 in homing and engraftment of HSCs and imply that reduced Shp2 levels may drive cells from cell cycle quiescence, potentially leading to HSC exhaustion, and accounts for the finding that animals lacking hematopoietic cell Shp2 expression are much more sensitive to treatment with the cell cycle-specific myeloablative treatment, 5-fluorour-acil [19■■].

To determine whether Shp2 functions in a cell autonomous manner in HSCs, or whether Shp2 function is needed in the microenvironment to regulate HSCs, reciprocal transplants were performed. Wild-type bone marrow (either unfractionated or LSK cells) transplanted into polyI : polyC-treated $Shp2^{flox/flox}$: Mx1-Cre-positive or $Shp2^{flox/flox}$: Mx1-Cre-negative (Feng model) lethally irradiated recipients produced similar levels of hematopoietic reconstitution [19■■]. Consistently, wild-type bone marrow transplanted into lethally irradiated Mx1-Cre-positive; $PTPN11^{flox/+}$ or Mx1-Cre-positive; $PTPN11^{flox/flox}$ animals (Neel model) followed by treatment with polyI : polyC also yielded similar levels of hematopoiesis with animals remaining healthy with normal blood counts for at least 20 weeks postpolyI : polyC [18■■]. These combined studies nicely corroborate that Shp2 functions in a cell autonomous manner in HSCs.

The molecular mechanism underlying how Shp2 modifies HSC and progenitor function has also been investigated. Zhu *et al*. [19■■] found that Kit expression is reduced in the bone marrow of $Shp2^{flox/flox}$: Mx1-Cre-positive animals, and further demonstrated using chromatin immunoprecipitation and luciferase studies that GATA2 binding to the *Kit* promoter and *Kit* promoter activity, respectively, are regulated by Shp2 expression. Chan *et al.* [18■■] examined the role of p53 in the increased apoptosis levels observed in Lin-negative Kitpositive and LSKCD150-positive cells from polyI : polyC-treated Mx1-Cre-positive; $PTPN11^{flox/flox}$ animals. A dominant negative p53 failed to inhibit upregulation of proapoptotic Noxa expression in LSK cells lacking Shp2 expression, suggesting that loss of Shp2 induces p53-independent apoptosis in HSC/progenitors [18■■]. Using Shp2 knockdown in 32Dcl3 cells, Zhang and Friedman [20] found reduced CCAT/enhancer binding protein α (C/EBPα) expressionat the RNA and protein levels and reduced granulopoietic differentiation, and further observed that knockdown of Erk reduced proliferation, but did not change C/EBPα protein expression or granulopoiesis, suggesting a unique Erk-independent role of Shp2 in granulopoiesis.

Shp2 IN HUMAN HEMATOPOIESIS

Most studies have utilized murine models to study Shp2 function in hematopoiesis; therefore, Li *et al*. [21■■] used a lentiviral system to infect human $CD34^+$ cells with shRNA targeted to Shp2 in order to better understand the role of Shp2 in human hematopoiesis. Consistent with findings using murine cells, human $CD34^+$ cells deficient in Shp2 have reduced growth factor-stimulated proliferation and survival and demonstrate reduced myeloid and erythroid cell differentiation compared with control cells [21■■]. Biochemically, loss of Shp2 in $CD34^+$ cells results in reduced growth factor-activated Erk, AKT, and JAK/STAT signaling and reduced expression of the antiapoptotic genes *MCL1* and *BCLXL* [21■■]. These studies are particularly relevant and timely given the aberrant Shp2 expression and function in human HSC-related diseases.

Shp2 IN HUMAN DISEASE AND LEUKEMOGENESIS

Given the critical role of Shp2 in HSC function and in human hematopoiesis, it is not surprising that dysregulated Shp2 function is commonly found in human myeloid leukemias. Shp2 was originally found to be affiliated with human disorder when approximately 50% of individuals with the congenital disorder Noonan syndrome were found to bear germline gain-of-function mutations in *PTPN11* [10]. Although individuals with Noonan syndrome demonstrate several congenital anomalies including cardiac defects, craniofacial abnormalities, and short stature, it was also noted that Noonan syndrome patients rarely develop the uncommon childhood leukemia, juvenile myelomonocytic leukemia (JMML). This observation led to the finding that children with sporadic JMML bear somatic gain-of-function *PTPN11* mutations in 35% of cases [22]. These gain-of-function mutations are typically point mutations in the N-SH2 (amino-terminal Src homology 2) domain or, less commonly, in the phosphatase domain, which prevent autoinhibition between the catalytic domain and the N-SH2 domain and render the protein constitutively active [5,10,22].

In addition to JMML, gain-of-function mutations in *PTPN11* have also been rarely associated with other hematologic malignancies including pediatric and adult acute myeloid leukemia (AML) [23–25], chronic myelomonocytic leukemia [26], B-cell acute lymphoblastic leukemia/lymphoma (B-ALL) [27], and pediatric and adult myelodysplastic syndrome (MDS) [26,28,29]. The biological differences underlying the relatively low frequency of PTPN11 mutations in most hematologic malignancies compared with the high frequency found in JMML is unclear; however, one hypothesis suggests that in AML and MDS, *PTPN11* mutations (leading to altered signal transduction pathways and increased proliferation) may need to work in a cooperative fashion with transcription factor mutations (leading to reduced cellular differentiation). Consistent with this notion, in AML and MDS, *PTPN11* mutations are not uncommonly found to be concurrent with recurrent leukemia-associated cytogenetic anomalies [23,30]. Consistently, the *PTPN11* gain-of-function mutant Shp2E76K has been shown to cooperate with overexpression of HoxA10 and with deficiency of interferon consensus sequence-binding protein in the induction of AML [31,32].

Although Shp2 mutations are uncommonly found in adult leukemias, Shp2 has been shown to be overexpressed at both the protein and RNA levels in several human AML cell lines and primary samples [33]. Upon immunoblot analyses, Xu *et al*. [33] detected two distinct Shp2 bands and, interestingly, the upper (tyrosine phosphorylated) band was preferentially expressed in both the membranous and nuclear fraction of leukemic cells. Additionally, increased Shp2 phosphorylation in primary leukemic cells correlated with hyperplastic bone marrow, suggesting that phospho-Shp2 levels are positively associated with high proliferative potential of leukemic bone marrow cells. Finally, using the Breakpoint Cluster Region – Abelson kinase-positive human cell line K562, which has a high expression of Shp2, reduction of Shp2 expression using antisense Shp2 onligonucleotides induced apoptosis and inhibited leukemic cell clonogenic growth [33].

The first human *PTPN11* mutation modeled in animals, generated in the laboratory of Dr Benjamin Neel, was engineered to constitutionally express the germline Noonan syndrome mutation, Shp2D61G [34]. Homozygous *PTPN11*$^{D61G/D61G}$ animals were embryonic lethal; however, approximately 50% heterozygous *PTPN11*$^{D61G/+}$ animals were viable. Surviving pups developed similar characteristics to individuals with Noonan syndrome including short stature, craniofacial abnormalities, cardiac defects, and indolent myeloproliferative disorder (MPD) [34] (Fig. 1b). Studies performed in the laboratory of Dr Cheng-Kui Qu using this animal model [35] demonstrated that LSK cells as well as the phenotypically defined progenitors CMP, GMP, and MEP in the PTPN11$^{D61G/+}$ animals were increased in both the bone marrow and spleen and that, functionally, HSCs from the PTPN11$^{D61G/+}$ animals demonstrated increased repopulating capacity compared with control cells [35] (Fig. 1b). Furthermore, transplantation of whole bone marrow or LSK cells was able to produce MPD in recipient animals (Fig. 1b), whereas transplantation of committed progenitors (CMP or GMP) failed to produce MPD [35]. In contrast, as described in detail below, animals conditionally expressing somatic gain-of-function Shp2 mutants (Shp2D61Y and Shp2E76K) in hematopoietic tissues demonstrated reduced bone marrow phenotypic HSCs and progenitors as well as reduced HSC function [36,37■■] (Fig. 1b, c, and d). These phenotypic distinctions may be due to the fact the Shp2D61G is a less severe mutation with lower phosphatase activity compared with Shp2D61Y and Shp2E76K [38], or may be due to the broader, global expression of Shp2D61G that may exert microenvironmental effects in the *PTPN11*$^{D61G/+}$ animals.

To simulate human JMML, Shp2D61Y or Shp2E76K (the two most common *PTPN11* mutations found in individuals with JMML) were retrovirally transduced into 5-fluorouracil-treated bone marrowcellsfrom Balb/c mice and transplanted into lethally irradiated syngeneic recipients. Approximately 50% of animals developed leukocytosis and splenomegaly followed by death between 6–7 months, and over 90% eventually succumbed to death by 12–18 months posttransplant [39]. In order to develop a more physiologic model of mutant Shp2-induced leukemia, a conditional knockin of *PTPN11*^{D61Y} was generated expressing mutant Shp2 under its endogenous promoter and crossed with Mx1-Cre-positive transgenic animals [36]. PolyI: polyC-induced Cre expression is able to remove a stop codon within the *PTPN11*^{D61Y} allele allowing gain-of-function Shp2D61Y to be expressed. PolyI : polyC-treated *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive mice had an average survival rate of 45

weeks and typically succumbed to MPD with marked leukocytosis and hepatosplenomegaly, whereas the control animals lived at least 75 weeks without any major indications of disease. In contrast to those found in the *PTPN11*$^{D61G/+}$ animals [35], the bone marrow LSK cells, long-term HSC (LT-HSC, LSK, FLK2^{-}, CD34^{-}), short-term HSC (ST-HSC, LSK, FLK2^{-}, CD34^{+}), and phenotypic progenitor populations were significantly reduced in the *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive animals (Fig. 1c). Interestingly, however, the numbers of splenic LSK cells as well as ST-HSC and multipotent progenitors (MPP, LSK, FLK2^{+}, CD34^{+}) were increased in *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive animals (Fig. 1c), suggesting mutant Shp2 in the bone marrow encourages cells from the quiescent bone marrow compartment to migrate and expand into the spleen. Transplantation of either bone marrow or spleen cells from diseased *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive animals into lethally irradiated syngeneic recipients revealed that long-term engraftment of these mutant HSCs failed to give rise to MPD in recipient animals [36] (Fig. 1c).

A conditional knockin of *PTPN11*^{E76K} was also generated, as Shp2E76K is the most common Shp2 mutation found in JMML [37■■]. *PTPN11*$^{E76K/+}$ animals were crossed with Mx1-Cre transgenic animals and polyI : polyC-treated *PTPN11*$^{E76K/+}$; Mx1-Cre-positive animals develop fully penetrant MPD. Very similar to the *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive animals, bone marrow LSK cells, LT-HSCs, ST-HSCs, and phenotypic progenitors were significantly reduced in the polyI : polyC-treated *PTPN11*$^{E76K/+}$; Mx1-Cre-positive mice compared with control mice (Fig. 1c). Additionally, the *PTPN11*$^{E76K/+}$ animals also demonstrate a significant increase in splenic LSK cells [37■■]. Both the *Ptpn11*$^{D61Y/+}$; Mx1-Cre-positive and *PTPN11*$^{E76K/+}$; Mx1-Cre-positive animals demonstrate reduced quiescence and increased cycling of phenotypic HSCs, suggesting that mutant Shp2-induced HSCs have accelerated differentiation, increased movement to the spleen, and expansion within the splenic microenvironment [36,37■■] (Fig. 1c and d). Although the conditional *PTPN11* knockout models [18■■,19■■] and the gain-of-function *PTPN11* knockin models (Shp2D61G, Shp2D61Y, and Shp2E76K) [34,36,37■■] all demonstrate increased HSC cycling (i.e., reduced HSC quiescence), the HSCs lacking Shp2 expression demonstrate elevated apoptosis, whereas HSCs expressing Shp2 gain-of-function mutants demonstrate significantly reduced apoptosis compared with control cells (Fig. 1a–d). Thus, although Shp2 deficiency and Shp2 gain-of-function mutants both cause reduced HSC quiescence, the difference in HSC apoptosis likely accounts for the dramatically different phenotypes of bone marrow failure and MPD development, respectively.

Distinct from the *PTPN11*$^{D61Y/+}$; Mx1-Cre-positive animals, the *PTPN11*$^{E76K/+}$; Mx1-Cre-positive animals commonly progressed from MPD to acute leukemias including AML, T-cell-ALL (T-ALL), and B-ALL [37■■] (Fig. 1d). As the *PTPN11*$^{E76K/+}$ animals progressed to full blown leukemias of various types, Xu *et al*. [37■■] next considered at which stage in hematopoiesis the initial leukemia-initiating stem cells (LSCs) became amplified contributing to leukemia. To test this, LSK cells from *PTPN11*$^{E76K/+}$ animals with MPD were transplanted into lethally irradiated recipients. Recipient animals all developed MPD, and 40% of them progressed to acute leukemia, suggesting that the LSCs are present within the stem cell/immature progenitor population. To determine whether the LSCs could also be derived from more mature, lineage-committed progenitors, transplants were performed using

cells bearing the knockin of the *PTPN11*^{E76K} allele specifically in GMPs, early-stage T cells, and early-stage B cells using LysM-Cre, LCK-Cre, and CD19-Cre, respectively. Between 40 and 50% of recipient animals developed AML, T-ALL, and B-ALL in a lineage-specific manner, and these leukemias persisted in lineage-specific primary and secondary transplant recipients. These findings suggest that HSC self-renewal programs are not required for Shp2E76K-induced transformation to LSCs.

The apparent functional difference between Shp2D61Y, which clearly induces MPD but fails to induce full-blown acute leukemia, and Shp2E76K, which is able to induce full-blown leukemia of various lineages, may be due to the increased phosphatase activity of Shp2E76K, which is known to have the highest phosphatase activity of the various gain-of-function Shp2 mutants tested [38]. However, although there is no direct comparison to Shp2D61Y-expressing cells, Xu *et al*. [37■■] also found that bone marrow and splenic cells from *PTPN11*$^{E76K/+}$; Mx1-Cre-positive animals commonly demonstrated aneuploidy. Because aneuploidy is known to be associated with mitosis abnormalities, they next examined the centrosomes of mutant-expressing *PTPN11*$^{E76K/+}$ cells and found amplification of and Shp2 localization to centrosomes in preleukemic *PTPN11*$^{D61Y/+}$ mice. These novel findings provide at least a partial explanation for nuclear localization of Shp2 previously observed in human leukemia samples [33]. Future studies to examine potential genetic instability induced by other gain-of-function Shp2 mutants as well as to further study the function of nuclear Shp2 will provide further insight into the mechanisms underlying mutant Shp2-induced leukemogenesis.

CONCLUSION

The protein tyrosine phosphatase Shp2 has been heavily studied over the past two decades and has been implicated in the congenital disorder Noonan syndrome as well as in JMML and less commonly in other hematologic malignancies. Further understanding of the molecular mechanisms of wild-type Shp2 and the pathogenesis of mutant Shp2 will provide the needed knowledge to develop and implement therapeutic strategies targeted to Shp2 in human disease.

Acknowledgments

This work was supported by the Riley Children’s Foundation and the U.S. National Institutes of Health (F31AG031648, SCN; CA134777, RJC). The authors gratefully acknowledge the administrative assistance of Linda S. Henson.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 337–338).

1. Freeman RM Jr, Plutzky J, Neel BG. Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of Drosophila corkscrew. Proc Natl Acad Sci U S A. 1992; 89:11239–11243. [PubMed: 1280823]
2. Feng GS, Hui CC, Pawson T. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science. 1993; 259:1607–1611. [PubMed: 8096088]
3. Feng GS. Shp-2 tyrosine phosphatase: signaling one cell or many. Exp Cell Res. 1999; 253:47–54. [PubMed: 10579910]
4. Neel BG, Gu H, Pao L. The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci. 2003; 28:284–293. [PubMed: 12826400]
5. Chan RJ, Feng GS. PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. Blood. 2007; 109:862–867. [PubMed: 17053061]
6. Xu D, Qu CK. Protein tyrosine phosphatases in the JAK/STAT pathway. Front Biosci. 2008; 13:4925–4932. [PubMed: 18508557]
7. Liu X, Qu CK. Protein tyrosine phosphatase SHP-2 (PTPN11) in hematopoiesis and leukemogenesis. J Signal Transduct. 2011:195239. [PubMed: 21799948]
8. Kontaridis MI, Swanson KD, David FS, et al. PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. J Biol Chem. 2006; 281:6785–6792. [PubMed: 16377799]
9. Stewart RA, Sanda T, Widlund HR, et al. Phosphatase-dependent and -independent functions of Shp2 in neural crest cells underlie LEOPARD syndrome pathogenesis. Dev Cell. 2010; 18:750–762. [PubMed: 20493809]
10. Tartaglia M, Mehler EL, Goldberg R, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet. 2001; 29:465–468. [PubMed: 11704759]
11. Qu CK, Shi ZQ, Shen R, et al. A deletion mutation in the SH2-N domain of Shp-2 severely suppresses hematopoietic cell development. Mol Cell Biol. 1997; 17:5499–5507. [PubMed: 9271425]
12. Qu CK, Yu WM, Azzarelli B, et al. Biased suppression of hematopoiesis and multiple developmental defects in chimeric mice containing Shp-2 mutant cells. Mol Cell Biol. 1998; 18:6075–6082. [PubMed: 9742124]
13. Chan RJ, Johnson SA, Li Y, et al. A definitive role of Shp-2 tyrosine phosphatase in mediating embryonic stem cell differentiation and hematopoiesis. Blood. 2003; 102:2074–2080. [PubMed: 12791646]
14. Chan RJ, Li Y, Hass MN, et al. Shp-2 heterozygous hematopoietic stem cells have deficient repopulating ability due to diminished self-renewal. Exp Hematol. 2006; 34:1230–1239. [PubMed: 16939816]
15. Saxton TM, Henkemeyer M, Gasca S, et al. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. EMBO J. 1997; 16:2352–2364. [PubMed: 9171349]
16. Zhang SQ, Yang W, Kontaridis MI, et al. Shp2 regulates SRC family kinase activity and Ras/Erk activation by controlling Csk recruitment. Mol Cell. 2004; 13:341–355. [PubMed: 14967142]
17. Zhang EE, Chapeau E, Hagihara K, Feng GS. Neuronal Shp2 tyrosine phosphatase controls energy balance and metabolism. Proc Natl Acad Sci U S A. 2004; 101:16064–16069. [PubMed: 15520383]
18■■. Chan G, Cheung LS, Yang W, et al. Essential role for Ptpn11 in survival of hematopoietic stem and progenitor cells. Blood. 2011; 117:4253–4261. Comprehensive analysis of Shp2 function in HSC-repopulating capacity using a model with complete Shp2 deficiency in the hematopoietic compartment (in contrast to the original studies that utilized an animal model expressing residual, truncated Shp2 protein). [PubMed: 21398220]
19■■. Zhu HH, Ji K, Alderson N, et al. Kit-Shp2-Kit signaling acts to maintain a functional hematopoietic stem and progenitor cell pool. Blood. 2011; 117:5350–5361. Comprehensive analysis of Shp2 function in HSC-repopulating capacity using a model with complete Shp2 deficiency in the hematopoietic compartment. First study demonstrating the necessity of Shp2 for HSC homing and early engraftment. [PubMed: 21450902]

20. Zhang L, Friedman AD. SHP2 tyrosine phosphatase stimulates CEBPA gene expression to mediate cytokine-dependent granulopoiesis. Blood. 2011; 118:2266–2274. [PubMed: 21725048]

21■■. Li L, Modi H, McDonald T, et al. A critical role for SHP2 in STAT5 activation and growth factor-mediated proliferation, survival, and differentiation of human CD34+ cells. Blood. 2011; 118:1504–1515. First study to examine Shp2 function in normal human hematopoiesis. [PubMed: 21670473]

22. Tartaglia M, Niemeyer CM, Fragale A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet. 2003; 34:148–150. [PubMed: 12717436]

23. Loh ML, Reynolds MG, Vattikuti S, et al. PTPN11 mutations in pediatric patients with acute myeloid leukemia: results from the Children's Cancer Group. Leukemia. 2004; 18:1831–1834. [PubMed: 15385933]

24. Bentires-Alj M, Paez JG, David FS, et al. Activating mutations of the Noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. Cancer Res. 2004; 64:8816–8820. [PubMed: 15604238]

25. Tartaglia M, Martinelli S, Iavarone I, et al. Somatic PTPN11 mutations in childhood acute myeloid leukaemia. Br J Haematol. 2005; 129:333–339. [PubMed: 15842656]

26. Loh ML, Martinelli S, Cordeddu V, et al. Acquired PTPN11 mutations occur rarely in adult patients with myelodysplastic syndromes and chronic myelomonocytic leukemia. Leuk Res. 2005; 29:459–462. [PubMed: 15725481]

27. Tartaglia M, Martinelli S, Cazzaniga G, et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. Blood. 2004; 104:307–313. [PubMed: 14982869]

28. Johan MF, Bowen DT, Frew ME, et al. Mutations in PTPN11 are uncommon in adult myelodysplastic syndromes and acute myeloid leukaemia. Br J Haematol. 2004; 124:843–844. [PubMed: 15009076]

29. Watkins F, Fidler C, Boultwood J, Wainscoat JS. Mutations in PTPN11 are rare in adult myelodysplastic syndromes and acute myeloid leukemia. Am J Hematol. 2004; 76:417. [PubMed: 15282682]

30. Christiansen DH, Desta F, Andersen MK, Pedersen-Bjergaard J. Mutations of the PTPN11 gene in therapy-related MDS and AML with rare balanced chromosome translocations. Genes Chromosomes Cancer. 2007; 46:517–521. [PubMed: 17330262]

31. Konieczna I, Horvath E, Wang H, et al. Constitutive activation of SHP2 in mice cooperates with ICSBP deficiency to accelerate progression to acute myeloid leukemia. J Clin Invest. 2008; 118:853–867. [PubMed: 18246201]

32. Wang H, Lindsey S, Konieczna I, et al. Constitutively active SHP2 cooperates with HoxA10 overexpression to induce acute myeloid leukemia. J Biol Chem. 2009; 284:2549–2567. [PubMed: 19022774]

33. Xu R, Yu Y, Zheng S, et al. Overexpression of Shp2 tyrosine phosphatase is implicated in leukemogenesis in adult human leukemia. Blood. 2005; 106:3142–3149. [PubMed: 16030196]

34. Araki T, Mohi MG, Ismat FA, et al. Mouse model of Noonan syndrome reveals cell type- and gene dosage-dependent effects of Ptpn11 mutation. Nat Med. 2004; 10:849–857. [PubMed: 15273746]

35. Xu D, Wang S, Yu WM, et al. A germline gain-of-function mutation in Ptpn11 (Shp-2) phosphatase induces myeloproliferative disease by aberrant activation of hematopoietic stem cells. Blood. 2010; 116:3611–3621. [PubMed: 20651068]

36. Chan G, Kalaitzidis D, Usenko T, et al. Leukemogenic Ptpn11 causes fatal myeloproliferative disorder via cell-autonomous effects on multiple stages of hematopoiesis. Blood. 2009; 113:4414–4424. [PubMed: 19179468]

37■■. Xu D, Liu X, Yu WM, et al. Nonlineage/stage-restricted effects of a gain-of-function mutation in tyrosine phosphatase Ptpn11 (Shp2) on malignant transformation of hematopoietic cells. J Exp Med. 2011; 208:1977–1988. First study indicating that the somatic *PTPN11* mutant Shp2E76Kis ableto induce acute leukemia of multiple lineages by transformation at both the HSC level and the hematopoietic progenitor level. The phenotypic difference between somatic Shp2D61Y-induced [18■■] and somatic Shp2E76K-induced disease in vivo may be due to the increased

phosphatase activity of Shp2 E76K compared with Shp2D61Y; however, this distinction has not yet been fully elucidated. [PubMed: 21930766]

38. Keilhack H, David FS, McGregor M, et al. Diverse biochemical properties of Shp2 mutants. Implications for disease phenotypes. J Biol Chem. 2005; 280:30984–30993. [PubMed: 15987685]
39. Mohi MG, Williams IR, Dearolf CR, et al. Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 (PTPN11) mutations. Cancer Cell. 2005; 7:179–191. [PubMed: 15710330]

KEY POINTS

- The protein tyrosine phosphatase Shp2 plays a crucial role in physiologic hematopoiesis and hematopoietic stem cell function.
- Loss of Shp2 in hematopoietic stem cells (HSCs) results in reduced quiescence and increased apoptosis, leading to substantially reduced HSC-repopulating activity.
- Endogenous expression of gain-of-function mutants Shp2D61Y and Shp2E76K reduces HSC-repopulating activity and promotes mobilization of phenotypic HSCs from the bone marrow to the spleen.
- Endogenous expression of gain-of-function Shp2D61Y induces myeloproliferative disease, but not acute leukemia.
- Endogenous expression of gain-of-function Shp2E76K induces myeloproliferative disease that progresses to acute leukemia of multiple lineages.

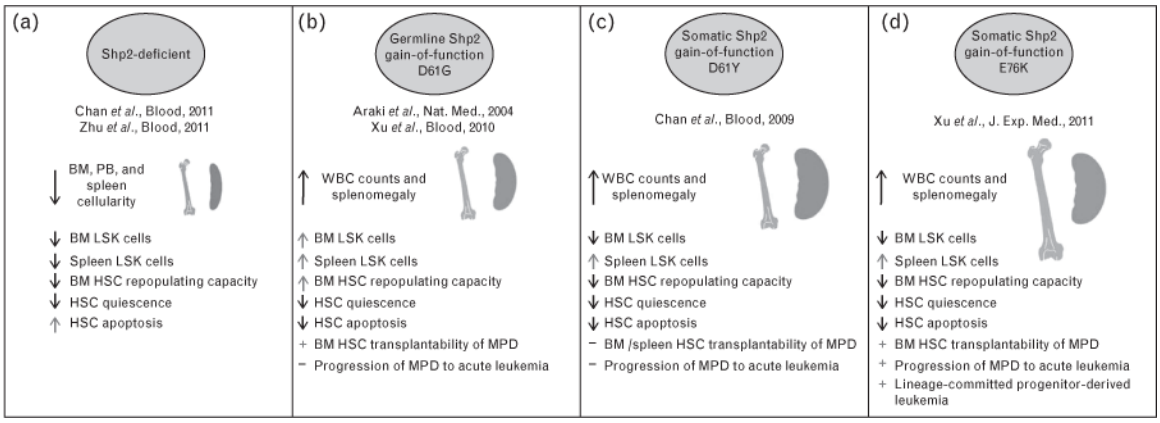

FIGURE 1.

Schematic diagram comparing the hematopoietic progenitor, hematopoietic stem cell, and leukemia transformation phenotypes of murine models bearing knockout of Shp2 (a), germline knockin of Shp2D61G (b), somatic knockin of Shp2D61Y (c), or somatic knockin of Shp2E76K (d). BM, bone marrow; HSC, hematopoietic stem cell; LSK, Lin-negative Sca1-positive Kit-positive; MPD; myeloproliferative disorder; PB, peripheral blood; WBC, white blood cell.